

Antimicrobial effectiveness of selected Indian spices

Surojit Bera*

Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India-144411

***Corresponding author:** Dr. Surojit Bera
E-mail: suromic@gmail.com
Phone: 1800 274 0615; Fax: +91-1824-506111

Abstract

In this study, the in vitro antimicrobial activity of four different solvent (ethanol, methanol, acetone, and aqueous) extracts of ten spices named Chilli, Turmeric, Dhania, Clove, Methi, Black pepper, Zira, Hing, Garlic and Fennel seed against some pathogenic bacteria (Escherichia coli (MTCC 569), Klebsiella pneumoniae (MTCC 4032), Bacillus cereus (MTCC 7166), Vibrio cholera (MTCC3906) and Shigella sp. (MTCC 9543)) were investigated. Among the selected species Zira, Hing, Garlic and Fennel seed show significance antimicrobial activity against all selected microbes in aqueous extracts. Further the species Turmeric, Dhania and Clove showed less effectiveness against Bacillus cereus and Shigella sp. in aqueous extract. However, the Chilli extract showed no significant activity for experimental organisms. Among all the spices Garlic showed the highest antimicrobial activity meaning highest zone of inhibition (08 mm) where as antibiotic, Ceftriaxone showed the activity 12 mm. The MIC of aqueous extract of Garlic was tested against all microorganisms. The dilution concentration (MIC) results exhibited significant at 1/40.

1. Introduction

World wide, communicable ailment is the predominant cause of death which responsible for tropical countries. For 1000 of years plants not have provided clean air to the human kind but also acted as a huge source for novel medicinal bioactive compounds which has significant contribution in human health [1-4].

Researchers have some basic reasons to be interested in the tropic of antimicrobial plant extracts. First, they are important in terms of over chemical use. Second, to deal

with the excessive prescribed antibiotic use. Third, to deal with the resistant microbes etc. [5-8].

This has commended the researches from entire globe for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents [9-12].

Bioactive chemicals ordinarily obtained from all plant structures and their strength differs fitting to the plant fragments. Parts containing the highest concentration of such compounds are preferred for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or combination. They inhibit the growth of microbes by binding their protein molecules together, breaking the peptide bonds of essential protein molecules and acting as chelating agents, altering their biochemical system, preventing utilization of available nutrients to the microorganisms. Some compounds also cause inflammation and lyses of microbial cells [13,14].

Spices and herbs are being a part of Indian traditional cooking procedures since ancient times. They are being used mainly due to their tremendous aroma and flavour. However from early nineteenth century they have been tested for their biological effectiveness like antibacterial, antiviral anticancer activities etc. [17,18]. Numerous small plants and spices are well documented for their strong antioxidant activity and also their lipid oxidation prevention power in in-vitro as well as in vivo studies.

Spices from different places over globe exhibit antibacterial and antifungal activity. Laboratory experiments conducted by different researchers from different countries indicated involvement of abundant puzzling food-borne pathogens with bioactive spice plants. Present study is an approach to deal with microbial pathogenesis with some common plant spices in extract.

2. Materials and methods

2.1. Spice material:

Sl No.	Common Name	Scientific Name	Fanily	Parts used
1.	Lavang, Clavus.	<i>Syzygium aromaticum</i>	Myrtaceae	Dried Flower Buds
2.	Coriandrum, Koriannon,	<i>Coriandrum sativum</i>	Apiaceae	Fruit, Leaves

3.	Koriandron, Dhona Chilli Pepper, Chilli, Chili and Chile	<i>Capsicum annuum</i>	Solanaceae	Fruit
4.	Indian Saffron, Halud	<i>Curcuma longa</i>	Zingiberaceae	Indian Saffron, Halud
5.	Pippali, Pfeffer, Poivre, Golmarich.	<i>Piper nigrum</i>	Piperaceae	Fruit, Seed
6.	Fenugreek, Mentya, Menthulu, Methi.	<i>Trigonella foenum-graecum</i>	Fabaceae	Seed
7.	Comino, Jeera, Zeera, Jilakarra	<i>Cuminum cyminum</i>	Apiaceae	Seeds
8.	Hing, Ingua, Devil's Dung, Kaayam	<i>Ferula assafoetida</i>	Apiaceae	Latex
9.	Velli ullipaaya, Lehsun, Rasun.	<i>Allium sativum</i>	Alliaceae	Bulb
10.	Saunf, Marathon, Ferula, Fenel, Fenol, Mourii.	<i>Fenniculum vulgare</i>	Apiaceae	Seeds, Leaves

2.2. Microorganisms

The pathogenic microorganisms used in this study included *Escherichia coli* (MTCC 569), *Klebsiella pneumoniae* (MTCC 4032), *Bacillus cereus* (MTCC 7166), *Vibrio cholera* (MTCC3906) and *Shigella sp.* (MTCC 9543).

2.3. Extraction

Five grams of powdered spices were extracted with 100 ml of different solvents (Water, Acetone, Ethanol, Butanol) maintained inside an incubator shaker for 24 h. Then filtration was done with the help of filter paper. The obtained supernatant was collected and stored in low temperature for further use.

2.4. Testing of Antimicrobial Activity

All extracts of all collected spice are tested against mentioned five organisms by standard Agar Well Diffusion (Kirby-Bauer method) and Minimum Inhibitory Concentration (MIC). Aqueous extracts of ten different spices used for Agar Well Diffusion techniques for initial screening. Then only one spice (Garlic), which given best zone of inhibition, is extracted using another three solvents like Acetone, Ethanol, Butanol and all four extracts are tested against *B. cereus* to determine their M I C. Finally, the aqueous extract of

Garlic is taken and tallied the activity with antibiotic, Ceftriaxone by measuring the zone of inhibition.

Agar Well Diffusion

Nutrient agar medium was used (Himedia) according to the maker's guidelines. 1.5% NB was taken and dissolved in warm deionised water and autoclaved. After autoclaving, media was cooled and poured in petri dishes. The agar medium should be allowed to cool to room temperature. Inoculum was spreaded on Nutrient Agar plates using a spreader. Using a 5 mm borer 5 wells are made in all the plates. Test samples were added and incubated for 18-24 hrs at 35-37°C aerobically in an incubator. [11].

Minimum Inhibitory Concentration (MIC)

Nutrient agar medium was used (Himedia) according to the maker's guidelines. After autoclaving, allow it to cool in a 45 to 50°C. 1 ml of diluted extract in to corresponding one tube. Add 1 ml of inoculum in to only test set. Thus different set is prepared for five organisms and five spices and their four different extracts. One tubes taken as blank, containing only broth. All tubes are incubated for 24 hrs at 337°C aerobically in a rotary shaker of an incubator. After 24 hours of incubation, each plate is examined for the MIC [20].

3. Results and discussion

The antimicrobial effectiveness of ten selected species in aqueous extracts against various microbes are summarizes in Table 2. Among the selected species Zira, Hing, Garlic and Mouri show significance antimicrobial activity against all selected microbes in aqueous extracts. Besides these, Methi and Black popper showed slightly significant results in terms of antibacterial activity against few bacteria (Table 2). Further the species Turmeric, Dhona and Clove are little effective against *Bacillus cereus* and *Shigella* sp in aqueous extract. However, the extracts of Chilli did not show any activity with the selected microbes. Among all the spices Garlic showed the highest antimicrobial activity meaning highest zone of inhibition (08 mm) whereas antibiotic, Ceftriaxone showed the activity 12 mm (Table-5). The aqueous extract of garlic showed the highest zone of inhibition among the four extracts (Water, Acetone, Ethanol and Butanol). Organic solvents like Acetone, Ethanol and Butanol also showed significance result. In this study, the antimicrobial activity of the crude aqueous extracts from 10 spices against five

bacteria were determined. The aqueous extract of the Garlic showed greater antibacterial activity than corresponding acetonic, alcoholic and butanolic extract (Tables 3) at the same concentrations. The current work explored that the use of aqueous formulation of plant extracts provides more consistent antibacterial effectiveness as associated to organic solvents. This results evidently directs that the antibacterial compounds of spices are readily soluble in aqueous solvents than organic solvents.

The MIC of aqueous extract of Garlic was tested against *B. cereus*. The dilution concentration (MIC) results (Table 4) exhibited significant at 1/40.

Table 1. Dilution protocol of plant extract (X=10, Y=9)

Dilution	Water (ml)	Extract (ml)
1/X	Y	1
1/2X	Y+0.5	0.5
1/4X	Y+0.75	0.25
1/8X	Y+0.875	0.125

Table 2. IZ (mm) of aqueous extracts of spices against different organisms
(Cork borer diameter – 05 mm)

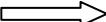
Name of the organisms  Name of the Spices 	<i>V. cholerae</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>Shigella sp.</i>	<i>S.pneumonia</i>
CLOVE	15	17	17	17	15
DHONIA	15	19	15	21	17
CHILI	15	17	15	19	17
TURMERI	15	17	17	19	17
BLACK PEPPER	17	17	19	21	19
METHI	19	21	21	18	16
ZIRRA	23	23	29	20	21
HING	23	24	23	20	22
GARLIC	28	37	25	31	29
MOURI	19	19	15	18	15

Table 3. IZ (mm) of different extracts of Garlic against different organisms
(Cork borer diameter – 05 mm)

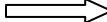
Name of the organisms  Name of the solvent 	<i>V. cholerae</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>Shigella sp.</i>	<i>S.pneumonia</i>
WATER	18	17	16	17	17
ETHANOL	11	13	11	12	07
ACETONE	13	11	13	14	15
BUTANOL	11	14	13	10	12

Table 4. MIC performance of Aqueous extracts of Garlic against *B. cereus* (Dilution, V/V)

Name of the Organism	Garlic			
	Aqueous			
	1/10	1/20	1/40	1/80
<i>B. cereus</i>	No Growth	No Growth	No Growth	Growth

Table 5. MIC performance of water extracts of Garlic against *B. cereus* (Dilution, V/V)

Sample	Concentration	Zone Of Inhibition
Ceftriaxone	50 mg/ml	29
Garlic extract	1/40 V/V	23

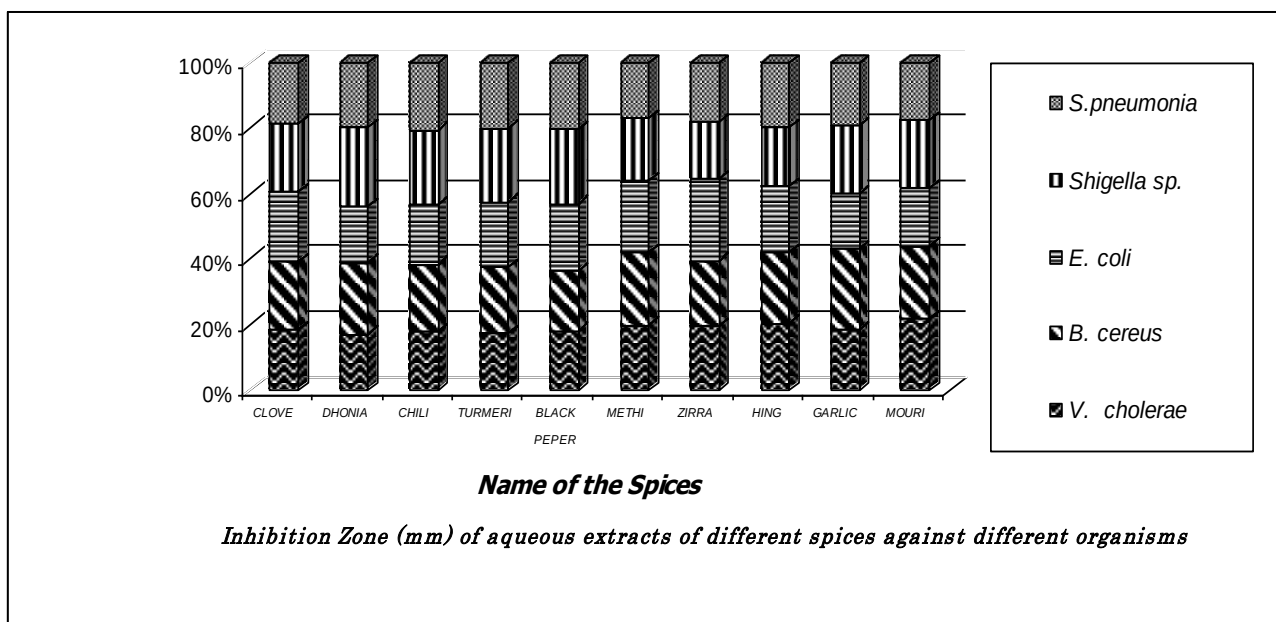


Figure 1. IZ (mm) of water extracts of different spices against different organisms.

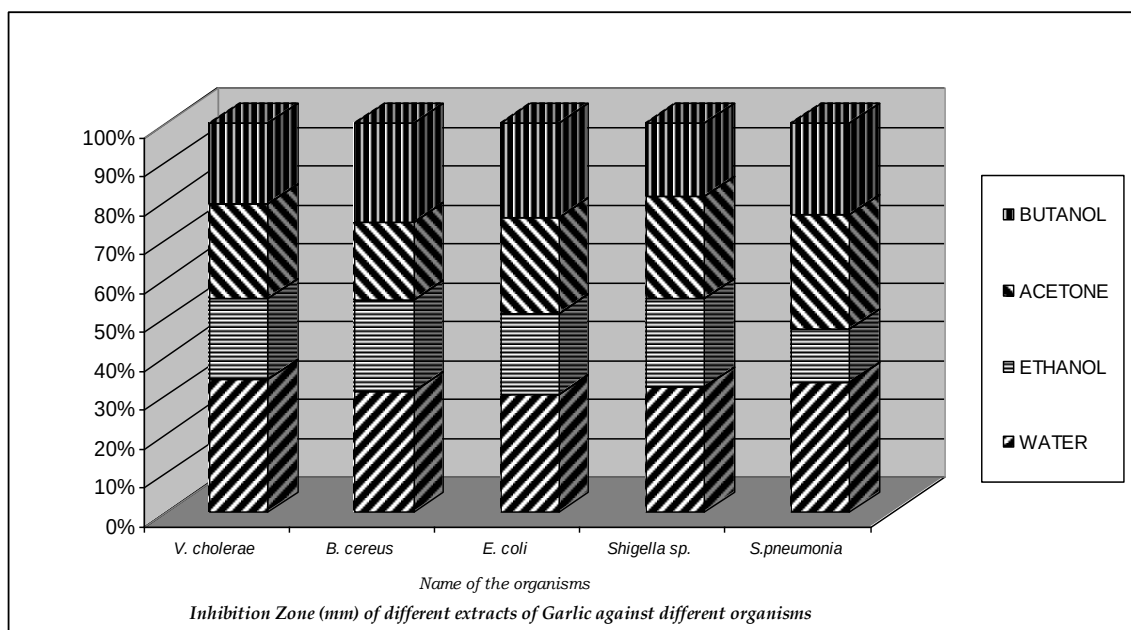


Figure 2. IZ (mm) of different extracts of Garlic against different organisms.

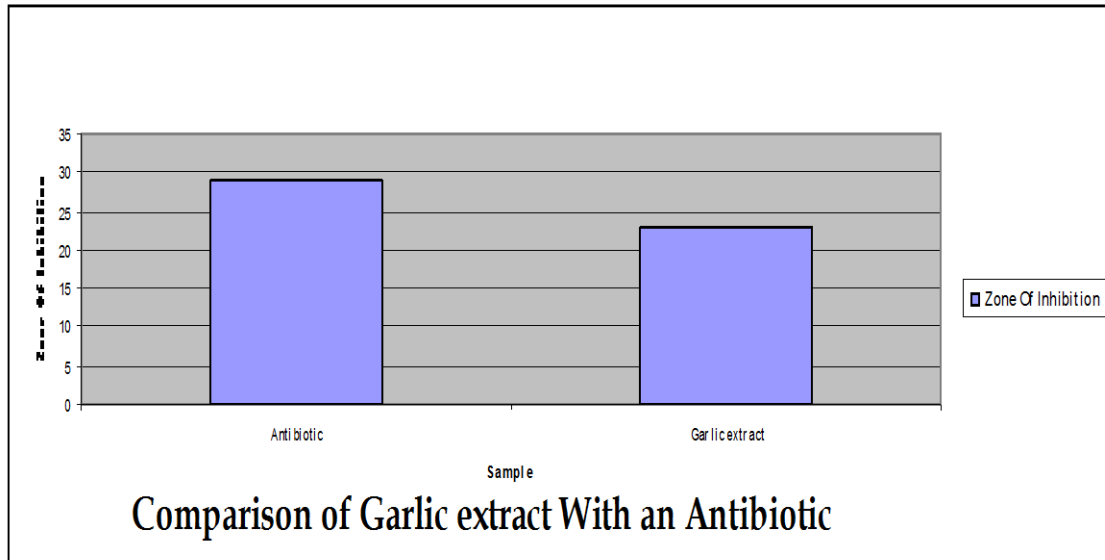


Figure 3. Comparison of Garlic extract with an Antibiotic.

4. Conclusion

The present study revealed that common spices used in Indian cooking science may have some tremendous potential of antimicrobial effectiveness beside their aroma and taste. Few from the selected spices showed potential activity against pathogens thus can be used as traditional medicine. Current study also supports the folkloric usage of the common spice and concludes that some of the plant extracts possess compounds with antibacterial properties. The garlic extract showed a potential agent for treatment of infectious diseases caused by organisms that are showing emergence of resistance to currently available antibiotics.

5. References:

- [1] Ahmedullah M., Nayar M.P., 1999. Red data book of Indian plants, vol. 4 (Peninsular India), Calcutta: Botanical Survey of India.
- [2] Akinyemi K. O., Oladapo O., Okwara E. C., Ibe C. C., Fasura A. K., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine, 5:6
- [3] Alma M.H., Mavi A., Yildirim A., Digrak M., Hirata T., 2003. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. Biological and Pharmaceutical Bulletin. 26: 1725–1729.
- [4] Almas K., 2001. The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatology Tropical. 24: 17–20.
- [5] Arora D.S., Kaur, J., 1999. Antimicrobial activity of spices. International Journal of Antimicrobial Agents. 12: 257–262.
- [6] Atindehou K.K., Kone M., Terreaux C., Traore D., Hostettmann K., Dosso M., 2002. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. Phytotherapy Research. 16: 497–502.
- [7] Barbour E.K., Al Sharif M., Sagherian V.K., Habre A.N., Talhouk R.S., Talhouk S.N., 2004. Screening of selected indigenous plants of Lebanon for antimicrobial activity. Journal of Ethnopharmacology. 93: 1–7.
- [8] Bera A.K. and Saha A., 1983. Antifungal activity of an aqueous extract of *Catharanthus roseus*. Zeitschrift fur pflanzen Krankheiten and pflanzen Schutz. 90:63.

- [9] Britto J.S. and Senthilkumar S., 2001. Antibacterial activity of *Solanum incanum* L. Leaf Extract. Asian Jr. of Microbiol. Biotech. & Env. Sc. 3(1-2): 65-66.
- [10] Deans S.G. and Ritchie G., 1987. Antibacterial properties of plant essential oils. Int. J. Food Microbiol. 5: 165–180.
- [11] Dev S., 1999. Ancient-modern concordance in Ayurvedic plants: some examples. Environmental Health Perspectives. 107: 783–789.
- [12] Duarte, M.C., Figueira, G.M., Sartoratto, A., Rehder, V.L., Delarmelina, C., 2005. Anti-*Candida* activity of Brazilian medicinal plants. Journal of Ethnopharmacology. 97: 305–311.
- [13] El-Seedi, H.R., Sata, N., Torssell, K.B., Nishiyama, S., 2002. New labdene diterpenes from *Eupatorium glutinosum*. Journal of Natural Products. 65: 728–729.
- [14] Fukai, T., Oku, Y., Hano, Y., Terada, S., 2004. Antimicrobial activities of hydrophobic 2-arylbenzofurans and an isoflavone against vancomycinresistant enterococci and methicillin-resistant *Staphylococcus aureus*. Planta Medica. 70: 685–687.
- [15] Garrod L. P., Lambert H. P., O'Gray F., 1975. Antibiotics and Chemotherapy, 4th Edn., Churchill Living Stone, Edinburgh, London, pp: 501-512.
- [16] Gislene G. F. N., Juliana L., Paulo C. F., Giuliana L. S., 2000. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic resistant Bacteria. Brazilian Journal of Microbiology. 31:247-256
- [17] Hargreaves L.L., Jarvis B., Rawlinson A.P. & Wood J.M. 1975. The antimicrobial e.ects of spices, herbs and extracts from these and other food plants. Scienti.c and Technical Surveys, British Food Manufacturing Industries Research Association. 88: 1–56.
- [18] Hirasa K. and Takemase M. 1993. Spice Science and Technology. Marcel Dekker, New York.
- [19] Jeevan Ram, A., Bhakshu, L.M., Venkata Raju, R.R., 2004. In vitro antimicrobial activity of certain medicinal plants from Eastern ghats, India, used for skin diseases. Journal of Ethnopharmacology. 90: 353–357.
- [20] Katerere, D.R., Gray, A.I., Nash, R.J., Waigh, R.D., 2003. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry. 63: 81–88.